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The Effect of Endotoxin on Abdominal Sympathetic Ganglia

By

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With 6 Figures

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Introduction

The notion is an old one that diarrheas of infectious origin might be caused by the action of a toxin on the neurovegetative periphery. However, their site of action within the autonomic nervous system remains in dispute as does the importance which should be ascribed to this mechanism.

Experimentally, *Moreau* (1868) produced what was called "paralytic hypersecretion of the succus entericus" by denervating a segment of intestine. *Cohnheim* (1882) drew an analogy from this experiment to clinical *cholera*, but emphasized that local intestinal reflexes alone sufficed to explain the phenomenon.

Mogilnizcky (1923), and *Staemmler* (1923) believed in a pathological-anatomical foundation for certain vegetative syndromes, and the former called attention to what was considered specific lesions in abdominal sympathetic ganglia of patients *with shigellosis*. The concept of the regional sympathetic ganglia as the target organ for certain "toxins" was given further experimental support by *Reilly* and coll. (1935) when they reproduced the intestinal lesion as well as the symptomatology of *typhoid fever* in animals by the injection of "endotoxin" into the regional abdominal ganglia. *Tinel* (1937) in his book has attached considerable significance to this mechanism.

Subsequently *Herzog* (1926) and *Terplan* (1926) refuted the specificity of lesions within sympathetic ganglia in various enteric infections and *Fulton* (1932) and others (*Ravina*, 1933; *Cushing*, 1932) at this time began to imply a central mechanism for certain visceral disturbances. *Penner* and *Klein* (1952) in recent years have postulated a CNS site of action for endotoxin on the basis of cross-circulation experiments in dogs. It would appear then as if the entire spectrum of possibilities has been covered.

The apparent confusion in the literature and our own concepts concerning intestinal infections have caused us to examine the autonomic nervous system in various diarrhea models. The present study is a revaluation of the possible

role played by endotoxin injected into the abdominal sympathetic ganglia of guinea pigs in the production of diarrhea and specific lesions within the neurovegetative periphery.

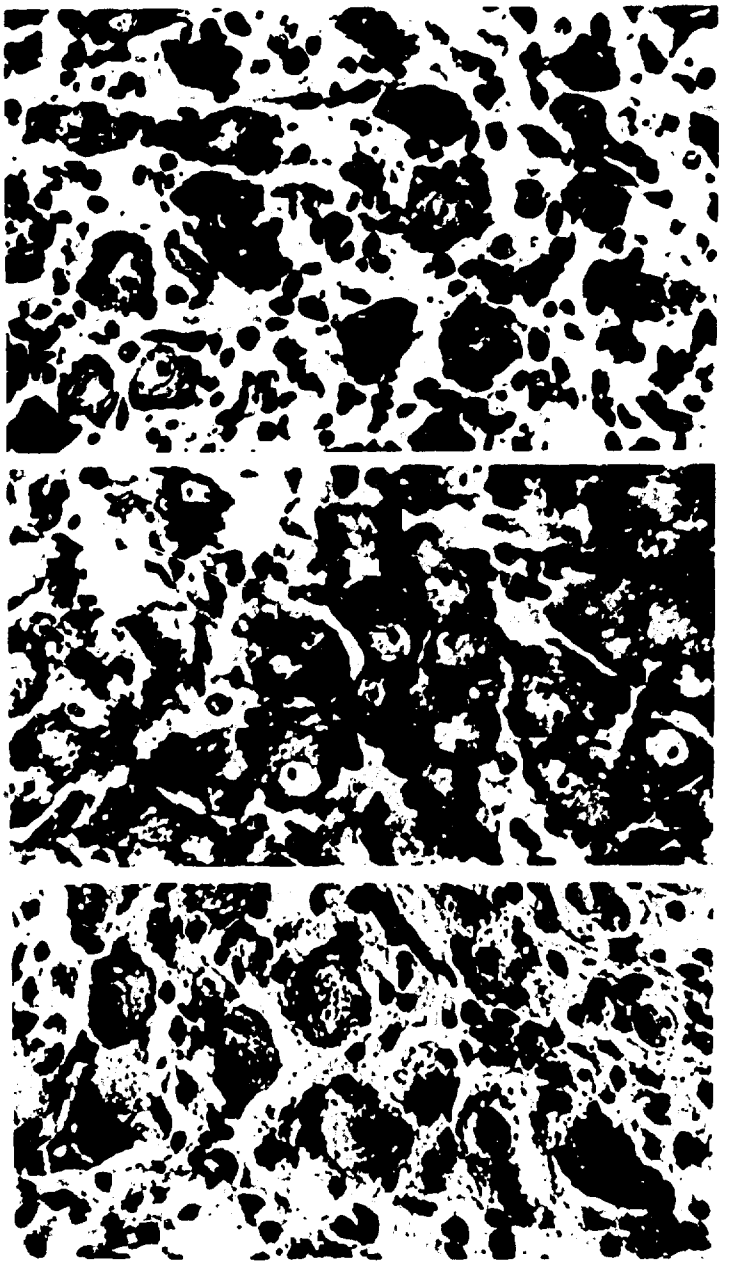

Fig. 1 c

Fig. 1 b

Fig. 1 a

Fig. 1. Abdominal sympathetic ganglion demonstrating fragmentation of nucleolus and clumping of chromatin within the nucleus 6 days after the injection of endotoxin (c). Nissl stain. ×384. As controls are seen normal (a and b) ganglia of guinea pig with H & E (× 355) and Nissl (× 365).

Material and Methods

Thirty-seven adult guinea pigs, Walter Reed strain, averaging 555 gms. body weight were utilized in this experiment. Three served as intact controls.

Twelve animals were given *Serratia marcescans* endotoxin (Difco) intraperitoneally through a midline laparotomy in a dosage of 1 ml. per 566 gms. body weight. Sixteen animals received 1 ml. of the same endotoxin per 554 gms. body weight into the periaortic ganglia which were approached through a left paramedian laparotomy. Another six animals had an equal volume of absolute ethanol or saline injected intraganglionicly.

Fig. 2. Typical central chromatolysis of sympathetic ganglion cells 36 hours after injection of endotoxin locally. One must be extremely careful in the interpretation of this type of change in sympathetic neurons, many of which normally have "clear" zones in the cytoplasm and eccentric nuclei. The neurons above, however, demonstrate complete clearing about the nucleus as well as nuclear swelling. H & E. × 640.

In order to ascertain the site of injection India ink was added to the endotoxin solution in three animals.

The guinea pigs were sacrificed at 24 to 48 hours, between the 4th and 6th day, and at three and four weeks. The abdominal sympathetic ganglia, spinal cord, brain and gastrointestinal tract were sampled and the tissues processed routinely for H & E and Cresyl Echt Violet stains. Pieces of gut were furthermore frozen and impregnated with ammoniacal silver carbonate or stained fresh with methylene blue.

The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

Results

Injection of endotoxin into the periaortic abdominal sympathetic ganglia results in a variety of pathological lesions within the ganglia without concomitant, demonstrable physiological alterations in the intestinal tract. The magnitude of our insult was identical in control and experimental groups (Table 1). None of the animals from either group developed diarrhea. Terminal diarrhea also was not observed. Animals surviving the first 24 hours after endotoxin injection appeared normal in all respects for the duration of the experiment, irrespective of the route of injection.

Table 1

Route of Injection	Animals	Average Weight	Dose ml. Weight gm.	Average Post Op. Weight Loss	OP Mortality	Diarrhea
Intra-ganglionic	12	603 gms.	1/554	50 gms.	13.6% by second day 33.3%	0
Intra-peritonial	22	454 gms.	1/566	46 gms.	16.6% by second day 25%	0

Following intraganglionic injection of endotoxin the earliest change in the ganglion cells was nucleolar fragmentation and clumping of nuclear chromatin (Fig. 1). In more severely affected cells typical central chromatolysis could be observed (Fig. 2). Eventually there was shrinkage of the cell away from its capsule. A number of animals, demonstrated a clear cut loss of neurons after two weeks, and proliferation of gliocytes (Fig. 3). Several ganglia furthermore displayed a noticeable proliferation of brown fat about the hilum of the ganglion, where a small amount of adipose tissue is found normally (Fig. 4 a).

In the periphery of the ganglia inflammatory cells, lymphocytes and macrophages, were noted early (Fig. 4 b). There was considerable and persistent hyperemia, often associated with periganglionic hemorrhages acutely.

No distinct pathological changes were noted in the intramural nervous system of the gut in these animals. The neurons of Auerbach's and Meissner's plexuses were normal. On occasion we observed a loss of granularity in the granular polydendrocytes, but otherwise the interstitial network appeared to be intact (Fig. 5). We found no ulcers.

The brain and cord were also normal. In all animals injected intraganglionicly with endotoxin pathological alterations could be found within

the ganglia; none of the control group showed alterations beyond infrequent cytoplasmic vacuolization or homogenization.

When absolute ethanol was injected into the abdominal ganglia in an effort to destroy these completely, changes similar to those described for endotoxin were observed, however, of greater magnitude (Fig. 6).

A mild degree of cellular change was noted in those regional ganglia into which saline rather than endotoxin was injected in equivalent amounts.

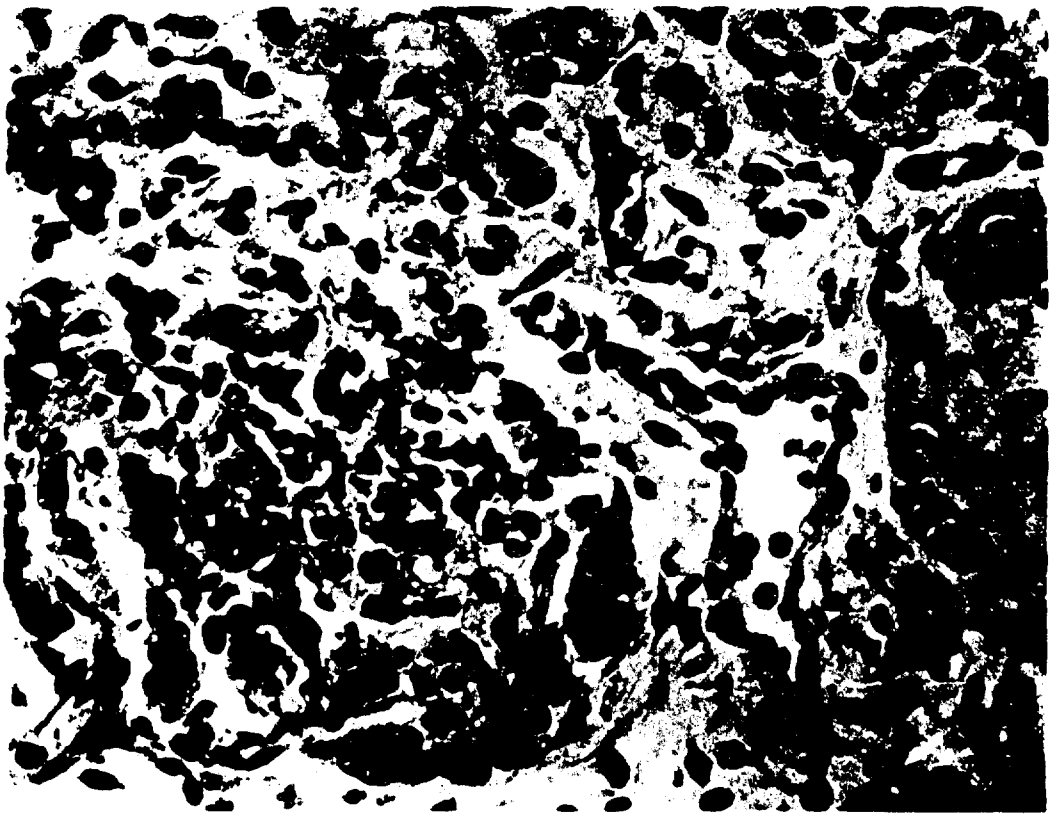

Fig. 5. Loss of neurons and active glial proliferation within a sympathetic ganglion 4 days after the injection of endotoxin. H & E. × 384.

Discussion

The present experiments underscore the lack of correlation between altered structure and detectable functional aberrations within the neurovegetative periphery (*Brüning*, 1958; *Herzog*, 1931). They furthermore do not support the importance of the role in infectious diarrheas formerly attributed to the regional sympathetic ganglia by French authors.

Endotoxin produces non-specific, toxic histological changes in the extramural ganglia, which can be reproduced by local injection of other noxious agents. The alcohol lesion within the abdominal sympathetic ganglia is neuronolytic such as was reported for the CNS by *Ghaimi* and *Hamby* (1962). While the endotoxin lesion might be considered largely reversible, it too

destroyed neurons. However, some of the vague cytoplasmic alterations were undoubtedly due to edema, rather than endotoxin per se. Cytoplasmic changes were caused by the local injection of an equal volume of saline as well, but this gave rise to little nuclear pathology. As emphasized by *Engelbrecht* (1951) one can be certain of true pathological changes within the vegetative nervous periphery, only when nuclear changes go hand in hand with alterations in the Nissl substance.

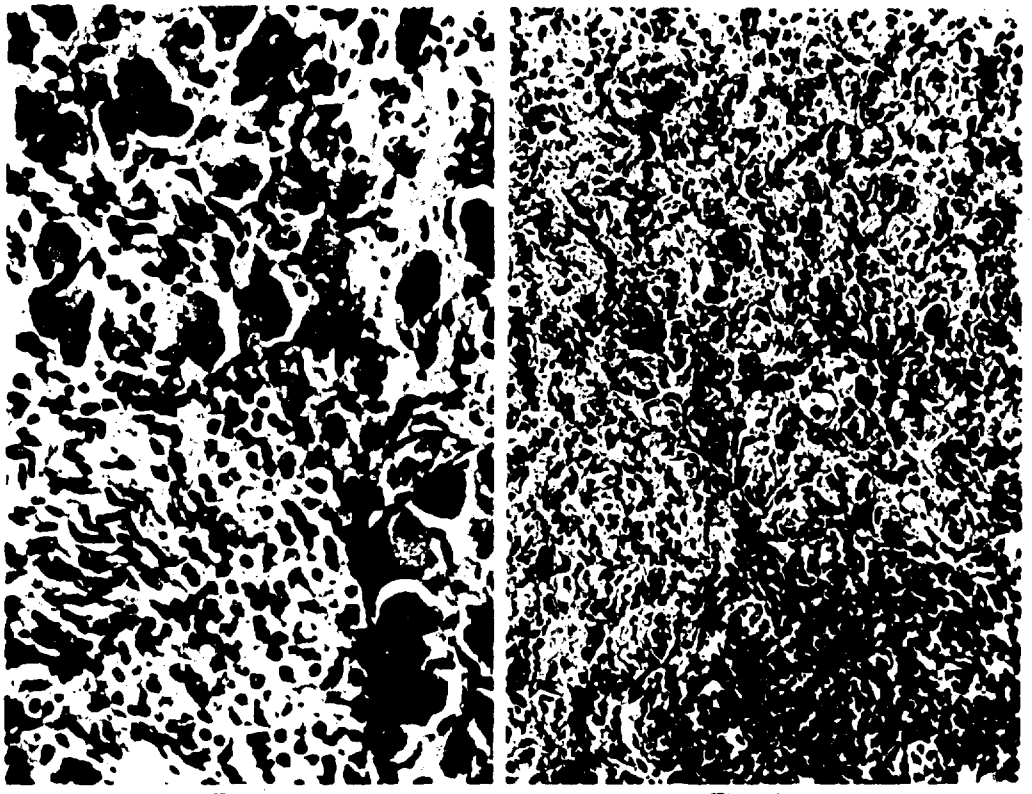

Fig. 4 a Fig. 4 b

Fig. 4. a) Hilum of abdominal sympathetic ganglion 16 days after the injection of endotoxin. There is an increased amount of brown fat in this ganglion. In addition, notice how the cells have shrunk away from their capsule. While this is seen as a frequent post-mortem artefact, it usually does not occur when these ganglia are fixed before 24 hours after death. b) Inflammatory infiltrate at the periphery of a ganglion 36 hours after local injection of endotoxin, utilizing aseptic technic. Early shrinkage of the neurons is noted. H & E. × 384.

Intravenous endotoxin administration has been shown to cause profuse diarrheas in reserpinized guinea pigs (*Kalas*, 1961). Reserpine gives what functionally amounts to a complete chemical sympathectomy (*Burn*, 1958). After catecholamine deplection, systemic endotoxin injection can, therefore, give rise to diarrhea.

On the other hand, extensive intramural lesions frequently involving Auerbach's plexus were demonstrated in all cases *with diarrhea*, in an analysis of human intestinal tuberculosis (*Leupold*, 1914).

In this context, it is important to view critically the difference between naturally occurring and experimental enteritis. Reilly was able to simulate

typhoid fever with a variety of stimuli, including Diphtheria exotoxin. Known neurotoxins, however, are rare among enteric pathogens.

We consider the neurovegetative periphery of the gastrointestinal tract relatively independent of central centers or extramural ganglia. The results presented here, as well as modern physiological evidence (*Kock*, 1959) bear us out. There are, no doubt, circumstances under which extrinsic regulation may become important, but endotoxin does not seem to be selectively aimed at

Fig. 5. Intact interstitial network of ileum with normal granular polydendrocytes and a well preserved primary fiber plexus in a guinea pig 16 days after endotoxin injection of the regional sympathetic ganglia. Methylene blue, × 192.

any extramural or central centers, even when applied directly to these. The so-called "Reilly phenomenon" can be reproduced in part at least by injection of endotoxin into the superior mesenteric artery (*Chamovitz*, 1962). This suggests an action on the intramural nervous system directly, without mediation through regional extramural vegetative ganglia.

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Summary

In the present study we have attempted to examine experimentally in 37 guinea pigs two points of longstanding controversy: Can diarrhea or any intestinal lesions be produced by the injection of endotoxin into the regional abdominal sympathetic ganglia, and secondly, does endotoxin evoke a specific pathological response in these ganglia? We have also attempted to correlate changes in the extramural ganglia, with alteration in the intramural nervous system.

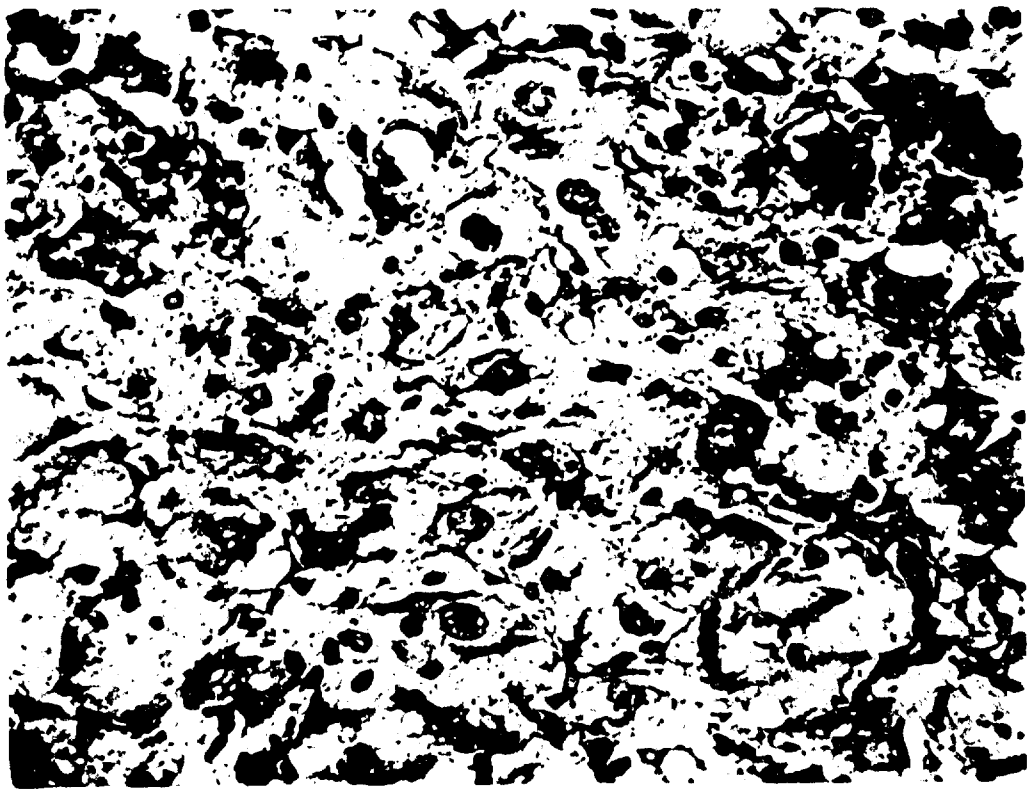

Fig. 6. Abdominal sympathetic ganglion four weeks after injection of absolute alcohol into the ganglion. There is persistent edema and severe neuronolytic changes. H & E. × 96.

When endotoxin was given intraganglionicly a variety of non-specific toxic lesions were noted in the ganglion cells as well as the gliocytes. Alcohol injection, in an effort to provoke an irritative effect, produced a more destructive lesion. No concomitant changes were observed in the gut. Diarrhea could not be precipitated in any of these animals, nor in their intraperitoneally injected controls, which had no histologic abnormalities in the regional abdominal sympathetic ganglia.

It is concluded, therefore, that the rôle of these ganglia should be minimized as a factor in enteritis, and that clinico-pathological correlation is difficult within the neurovegetative periphery.

Zusammenfassung

Diese Studie wurde von unserem Interesse an der Rolle, die das vegetative Nervensystem in verschiedenen Formen von Diarrhöe spielt, angeregt. Insbesondere wollten wir zur Klärung der Bedeutung des Endotoxin in diesem Zusammenhang

beitragen. Da menschliches Sektionsmaterial zur Beantwortung dieser Frage ungeeignet ist, haben wir experimentell bei Meerschweinchen untersucht, welche Wirkung das Endotoxin hat, das direkt in die Abdominalganglien eingespritzt wurde. Wir wollten feststellen, ob das so injizierte Endotoxin Durchfall oder pathologische Veränderungen an der Darminnervation hervorruft, und welche Veränderungen diese Injektion an den Sympaticusganglien selbst verursacht.

Unsere Untersuchungen haben ergeben, daß nach intraganglionärer Injektion von Endotoxin eine Reihe von allgemein-toxischen Veränderungen in den Ganglienzellen und Gliazellen der Sympaticusganglien zu beobachten sind; nur wenige Neuronen verfallen der Pyknose. Die neurovegetative Peripherie des Darmes blieb unverändert auch bei intraganglionären Äthanolinjektionen, die schwerere pathologische Veränderungen in den Ganglien erzeugten. Intraganglionäre Endotoxin- oder Äthanoleinspritzung verursachte bei keinem Meerschweinchen Diarrhöe.

Wir dürfen deshalb den Schluß ziehen, daß in diesem experimentellen Modell morphologische Veränderungen in den sympathischen Abdominalganglien keine Beziehung zur Entstehung von Diarrhöe haben; auch konnten wir keine, für Endotoxin spezifischen pathologischen Ganglienzellveränderungen feststellen.

Résumé

Cette étude a trait à une expérience pratiquée sur des cobayes pour éclaircir deux problèmes assez anciens: 1. La diarrhée ou d'autres lésions intestinales peuvent-elles être produites par l'injection d'endotoxine au voisinage des ganglions sympathiques abdominaux? 2. La réaction pathologique ganglionnaire est-elle spécifique de l'endotoxine? En même temps nous voulions établir un rapport entre les altérations pathologiques des ganglions extramuraux et celles des ganglions intestinaux intramuraux.

Après l'introduction intraganglionnaire de l'endotoxine, une variété de lésions non spécifiques apparaît dans les neurones et les gliocytes aussi. L'injection de l'alcool est encore plus destructive; il ne produit pas un effet irritatif mais des altérations vraiment neuronolytiques.

L'atteinte des ganglions sympathiques abdominaux ne touchait pas les ganglions intramuraux de l'intestin. Nous n'avons pas réussi à provoquer une diarrhée, ni chez les animaux d'expérience, ni chez les contrôles qui avaient reçu l'endotoxine dans le péritoine.

Nous concluons alors que l'endotoxine inoculée par voie intraganglionnaire n'engendre ni altérations pathologiques dans l'intestin, ni diarrhée. Par conséquent il faut minimiser la contribution des ganglions sympathiques abdominaux en diarrhée.

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